# X-ray Radiation Effects on

# Germination and Growth of *Echinacea angustifolia*

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## Introduction

Radiation exposure can affect organisms in many different ways. Out of all of the types of radiation, x-rays are the form that most people are exposed to outside of solar (gamma) radiation. X-ray radiation occupies the 0.01 – 10nm wave length range of the electromagnetic spectrum. Although the effects of x-ray radiation have been observed in humans from medical practices there is little information known about the effects of x-ray radiation on plants because x-ray technology is a relatively new development for plant studies. Radiation is known to aide in plant growth to a certain extent, but after a certain dose level the radiation harms the plant and may be lethal.[1,2] For example, radiation in doses from 200r to 1,000r (in Alders) and 75r to 300r (in Norway spruce) help plant growth, but above dose level 5,000r (Alders) and 600r (Norway spruce) the radiation harms the plant and is sometimes fatal.[3] Since x-ray technology applied to plant studies is relatively new there is little primary research that can be found on the topic. Most of the studies of radiation that have been conducted focus on gamma radiation not x-ray radiation; the few studies that do analyze x-ray radiation are relatively old, dating back to 1936.

It is important to research the effects of x-ray radiation because there are a number of institutions that are using x-ray machines to evaluate seed characteristics, and it is critical to know what effects or how x-ray radiation might change such characteristics, including seedling viability and flower formation. Research that has been conducted has only examined a small range of plant species leaving a vast number of species available to study. Purple Coneflower, *Echinacea angustifolia* is one of these species. This prairie flower was common, but its populations are dwindling due to degraded habit and can now be found in fragment prairies in the Northern United States. The decrease in *E. angustifolia* populations made these plants a target for research by population biology scientists.[4] The great interest in the plant means that it is fairly well studied, yet there is no information about the effects x-ray radiation has on the plant. The Echinacea Project plans to use x-rays do determine whether or not an achene has been fertilized. This experiment examines effects of x-ray radiation on *E. angustifolia* germination and growth, over a vast range (10-35kV) of energy levels to determine effcts of radiation on seed germination and growth, and the lethal dose for *E. angustifolia*.

## Methods

*Achene Selection and Irradiation*

On March 23, 2012 I selected about 880 achenes at random from a jar of random achenes collected between 2009 and 2011. On March 30, 2012 I took a sample of 50 achenes at random from the population of about 880 and weighed them to determine the ratio of fertilized (full) achenes and unfertilized (empty) achenes (34 of the 50 were fertilized, almost 68 percent). I then placed the sample of 50 back in the population of 880 and then counted, sorted, and randomized the achenes into 2.5x3.5 coin envelopes using a Seedburo 801 Count-A-Pack counting machine and selecting envelopes at random from an already shuffled order. The envelopes were pre-assigned dose treatments and corresponding batch numbers, labeled accordingly (there were 27 treatments (and batches), envelopes number from 1-27), and then shuffled for number randomization. With the counting machine I set the machine to place 32 achenes in each envelope.

On April 2, 2012 I irradiated each envelope individually according to its pre-determined dosage using a Faxitron MX-20 model x-ray machine with a current of 0.3mA at a source to object distance of 57.2cm for 10 seconds. I left one envelope as a control not irradiated and then x-rayed the others increasing the voltage (kV) in increments of one for each batch starting at 10kV (the lowest possible voltage) and ending with 35kV (the highest possible voltage). The number of batches is equal to the number of all possible voltage settings. Table 1 expresses estimated radiation dose in Roentgens per hour. The radiation dose was estimated based on the information provided by the x-ray machine manufacturer.[5]

Table 1

Estimated Radiation Dose for batches 1-27.

| Batch/Dish | Voltage (kV) | Estimated Radiation Dose (R/hr) |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 10 | 40.0 |
| 3 | 11 | 59.2 |
| 4 | 12 | 80.4 |
| 5 | 13 | 104.8 |
| 6 | 14 | 132.8 |
| 7 | 15 | 170.4 |
| 8 | 16 | 210.6 |
| 9 | 17 | 247.0 |
| 10 | 18 | 292.6 |
| 11 | 19 | 333.0 |
| 12 | 20 | 376.1 |

| | | |
|---|---|---|
| 13 | 21 | 420.2 |
| 14 | 22 | 465.2 |
| 15 | 23 | 521.6 |
| 16 | 24 | 568.6 |
| 17 | 25 | 618.2 |
| 18 | 26 | 667.4 |
| 19 | 27 | 729.4 |
| 20 | 28 | 778.8 |
| 21 | 29 | 833.0 |
| 22 | 30 | 906 |
| 23 | 31 | 950 |
| 24 | 32 | 1004 |
| 25 | 33 | 1062 |
| 26 | 34 | 1112 |
| 27 | 35 | 1140 |

*Pre-germination*

On April 2, 2012 the achenes were taken from their envelopes and placed into Petri dishes (dish). I had placed a piece of numbered blotter paper in each dish as a media for moisture retention. Each piece of blotter paper was moistened with 1,700µl of Florel solution so that the blotter paper was completely saturated. The achenes were then placed into a refrigerator with low light at about 4°C for about 14 days, with seeds randomly rotated almost every day, remoistening with 500µl of Florel on April 6, 9, and 16, 2012. This pre-germination and germination process is standard procedure for the Echinacea Project, which was developed based in the findings of S.M.J Feghahati and R.N. Reese in 1994.[6]

*Germination*

On April 15, 2012 the achenes were placed into a growth chamber at 25°C, 60% humidity (%RH) with 0.002 µMOL of light to begin the germination process. On April 16, 2012 I began to plant the seedlings as they sprouted into five 200 plug trays full of soil saturated with water. On this first day of planting 192 seedlings were plated. The plug trays were placed in the growth chambers at the same settings. In the growth chamber the settings will not change for the remainder of the germination process. Upon placement in the growth chamber each tray is assigned a position at random and was continually rotated in this fashion for the remainder of the experiment (random tray rotation is done almost every day till the end even if no sprouts are planted). On April 18, 2012 I watered each tray to complete saturation (I watered to saturation each time before planting for the remainder of the germination process) and then continued planting seedlings, 275 seedlings were planted bring the total number of seedlings planted to 467. I remoistened the dishes with 500µl of Florel. On April 20, 2012 seedling planting continued. Only 23 seedlings were planted bringing the total to 490 planted seedlings. I remoistened the dishes with 500µl of Florel. On April 23, 2012 planting continued. Just 22 seedlings were planted bringing the total to 512 seedlings planted. Again, I remoistened the dishes with 500µl of Florel. On April 27, 2012 planting continued. Only 1 seedling was planted bringing the total to 513 seedlings planted. I remoistened the dishes with 1000µl of Florel. On April 30, 2012 planting continued, but there was only 1 sprout to plant, making the total of 514 seedlings planted.

*Measurements*

On April 30, 2012 I measured the first batch of 14 day old sprouts. Measurements were taken from the cotyledons to the tip of the true leaf using a ruler (taken in millimeters). On May 2, 2012 and May 4, 2012 measurement of the sprouts continued on the $14^{th}$ days. On May 7, 2012 the measurements were taken even for the two batches that were not yet 14 days old. On May 31, 2012 concluding measurements were taken for all plants at about a month old (35 days).

*Unfertilized to Fertilized Ratio*

April 30, 2012 was the last day of planting; dishes with their remaining achenes were x-rayed again to determine how many achenes were actually fertilized or unfertilized. By looking at the images I could clearly see which achenes were fertilized and mostly tell which achenes were not fertilized. To be precise I counted the total number of achenes left in a dish and subtracted the number of fertilized achenes, which I knew for sure, from the total number of achenes in the dish. I then calculated the total number of fertilized achenes and the total number of unfertilized achenes. I did this calculation by adding the number of unplanted achenes to the total number of planted achenes to get the total number of fertilized achenes, and then I subtracted this number from the total number of seeds that were used in this experiment to get the total number of unfertilized achenes; with these two numbers together I had the total ratio of fertilized achenes to unfertilized achenes.

*Data and Statistical Analysis*

Data analysis and statistical analysis was conducted in Microsoft Excel. To examine the linear regression of each graph I used the “linest” (linear estimate) function. The important result of this function yielded the slope, degrees of freedom, and the f-value of the data; these values were among a few other statistical values that were not critical to this experiments analysis. Using the f-value I then conducted an “f-dist” (f-distribution) function to determine the p-value of each data set.

## Results

In all, 86 percent of the achenes germinated. As shown in figure 1, dish number two has the greatest percent of germinated seeds (96%) while dish twelve had the lowest percent of germinated seeds (72%). The percent of achenes germinated had the least evidence to support an effect of x-ray dose of all traits measured ($p=0.437$). Based on the percent germination from each batch compared to the level of radiation for each treatment there is a slightly negative slope (-0.003), but the relationship is not tight enough to reach statistical significance.

The total amount of growth by all measurable plants after 14 days was 3,075mm (7,939 mm after 35 days; of this height over 90 percent of the plants were planted on the first two days of planting. As shown in figure 2, the dish with the most growth was dish ten (372mm) and the dish with the least amount of growth was dish twenty-four (27mm). The total seed growth per dish compared to radiation dose yielded a p-value of p=0.114, though it is not statistically significant it shows a best-fit regression line with a fairly strong negative slope (-0.062). From the graph (figure 2) it appears that if the trend continues a decrease in plant growth from 2-2.5mm will occur for every 1,000 R/hr of radiation. As can be seen in figure 4, the dish with the most growth after 35 days was dish two (585mm) and the dish with the least amount of growth was dish twenty-four (43mm). The total seed growth per dish compared to radiation dose yielded a p-value of p= 0.042, this is statistically significant and it shows a best-fit regression line with a strong negative slope (-0.129). From the graph (figure 4) it appears that if the trend continues a decrease in plant growth from 3-3.5mm will occur for every 1,000 R/hr of radiation.

The average growth per dish compared to radiation dose graph (figure 3) closely resembled the graph for the total growth per dish (figure 2). Because figure 3 takes into account the measurements of zero this more accurately reflects total growth per dish graph. The p-value for the average growth data was p= 0.116. This p-value is close to the p-value of the total growth per dish data. The slope for this graph (-0.003) is closer to the slope of the percent germination per dish graph (figure 1) than the total growth per dish graph. The slope for this graph, though, suggests even more of a negative trend than the first graph does. As shown in figure 5, after 35 days of growth the slope (-0.005) of average growth per dish does not vary that much from the 14 days average growth per dish slope; nor does the 35 days p-value (p = 0.127) differ significantly from the 14 days p-value. The slope of this graph further suggests an even more negative trend than previously observed.

Overall, the only finding that successfully rejects the null hypothesis is the total growth per dish after 35 days of growth. Each further finding of data across the fields of percent germination, total germination per dish, and average germination per dish has failed to reject the null hypotheses. The result in figure 4 demonstrates that overtime increasing levels of radiation may have an effect on individual plant growth. Although the rest data was statistically insignificant this result means that the levels of radiation that the achenes were exposed to may not be sufficient to have any effect, and what little effect it does have (if any) is so small that it would require an experiment with larger sample size to quantify the effect.

**Discussion**

As a whole, this experiment has not fully supported the hypotheses that x-ray radiation affects seed germination or growth nor has it entirely rejected the null hypothesis that radiation has not effect on seed germination or growth of *Echinacea* *angustifolia*. Even though the data is not statistically significant, with the exception of figure 4 results, the data suggests that what effects x-ray radiation has on *E. angustifolia* are for the most part negligible. Based on the linear regression of percent germination to radiation dose's p-value (p=0.436981) and graph (figure 1) it can be concluded that there is almost no effect x-ray radiation has on seed germination. The greatest effects of radiation is seen after 35 days of growth in the total growth per dish result with the only statistically significant p-value (p= 0.042021). Further effects of radiation that can be seen (though small it is) are depicted in the total growth per dish (14 days), average growth per dish (14 days), and the average growth per dish (35 days) data. With lower p-values (p=0.114412, p= 0.115915, and p= 0.127413, respectively) and the more noticeable negative slope in the graphs (figure 2, figure 3, and figure 5).

The results of this experiment are somewhat surprising given that in previous research on other species radiation has more of an adverse effect on the plants; although different plant species do respond differently to different variables. [7] One of the primary differences between this experiment and previous research is that the machine that this experiment utilized was designed for x-raying achenes, while the other researcher's radiation source may be much more lethal. This difference raises one of the few parts of this experiment that would be changed in the future. If the source of radiation was not designed for seeds the results may have been different. It would also be a good idea to increase the sample size of the experiment and to rinse the achenes in bleach to prevent the growth of mold on the achenes (which may yield nonviable achenes). Radiation has numerous different everyday sources including: solar radiation, airplanes, power plants, housing material, television, digital watches, false teeth, and even food. In light of how much radiation the achenes in this experiment were exposed to (per hour (R/hr)) the majority of the achenes are being irradiated with more radiation than most people are exposed to in a year (about 366mrads per year, or only 1mrads per day).[8,9]

Although there have not been x-ray studies on *E. angustifolia* other radiation experiments have been conducted with other plant species. Since Gamma radiation is the most common form of radiation in everyday lives it has been studied fairly extensively. The radiation levels are often high and a lethal dose can usually be administered. In these experiments the trend has been that the radiation aids plant growth to a certain extent, then it begins to harm the plant, and even kill it entirely. [10,11] Compared to my experiment the radiation levels that I was able to manipulate were rather low because the x-ray machine was designed for the x-raying of seeds. With this in mind the levels of radiation were not great enough to completely kill the plant.

There are limitations to doing a short-term study on a long-lived organism; for example the limitation of not having the time to see if the radiation affects the plant later in time, the radiation may shorten its longevity or weaken its defenses to help it live longer. The long-term of the effects of radiation harken future studies. Also, it would be important to examine possible genetic effects of x-ray radiation in *E. angustifolia* in future studies. Another study would be to analyze the effects of x-ray radiation on germination comparing *E. angustifolia* to other species. An additional future study would be to use x-ray radiation to simulate the amount of cosmic radiation *E. angustifolia* receives over time and examine character changes. More studies can be conducted on how radiation affects other characteristics of *E. angustifolia* such as disk and ray floret formation, cotyledon formation, and radicle formation, in all there is a significant amount of future studies open to investigation regarding x-ray radiation and its interaction with *E. angustifolia*

**Acknowledgements**

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## Tables and Figures

**Table 1**

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| 22 | 30 | 906 |
| 23 | 31 | 950 |
| 24 | 32 | 1004 |
| 25 | 33 | 1062 |
| 26 | 34 | 1112 |
| 27 | 35 | 1140 |

**Figure 1**

The percent of germinated seeds per dish compared to the radiation dose level. There is a slightly negative slope (-0.002 % germinated per (R/hr)), but it is not statistically significant according to a linear regression ($p = 0.437$, $N = 27$ dishes).

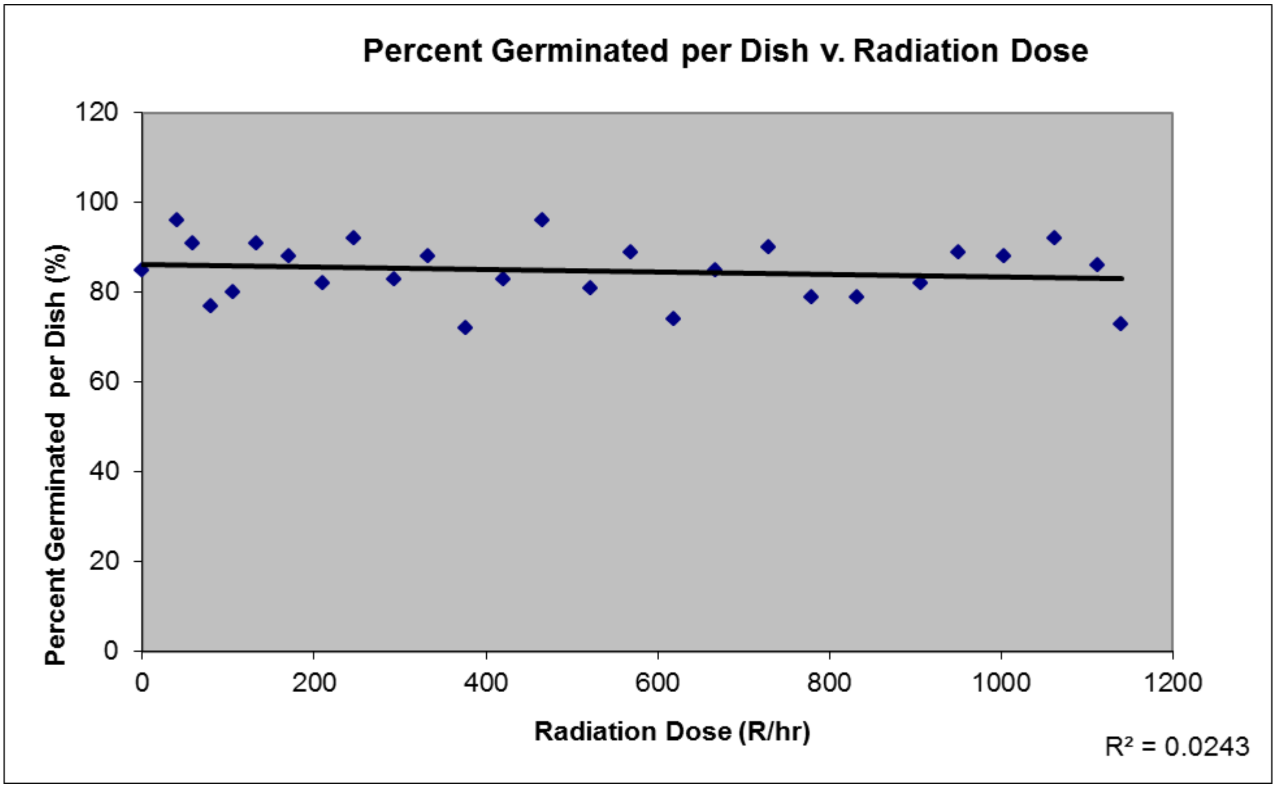


**Figure 2**

The total seedling growth per dish compared to the dose of radiation after 14 days of growth. There is a negative trend (-0.062 total growth in mm per (R/hr)), but it is not statistically significant according to a linear regression ($p = 0.114$, N = 27 dishes).

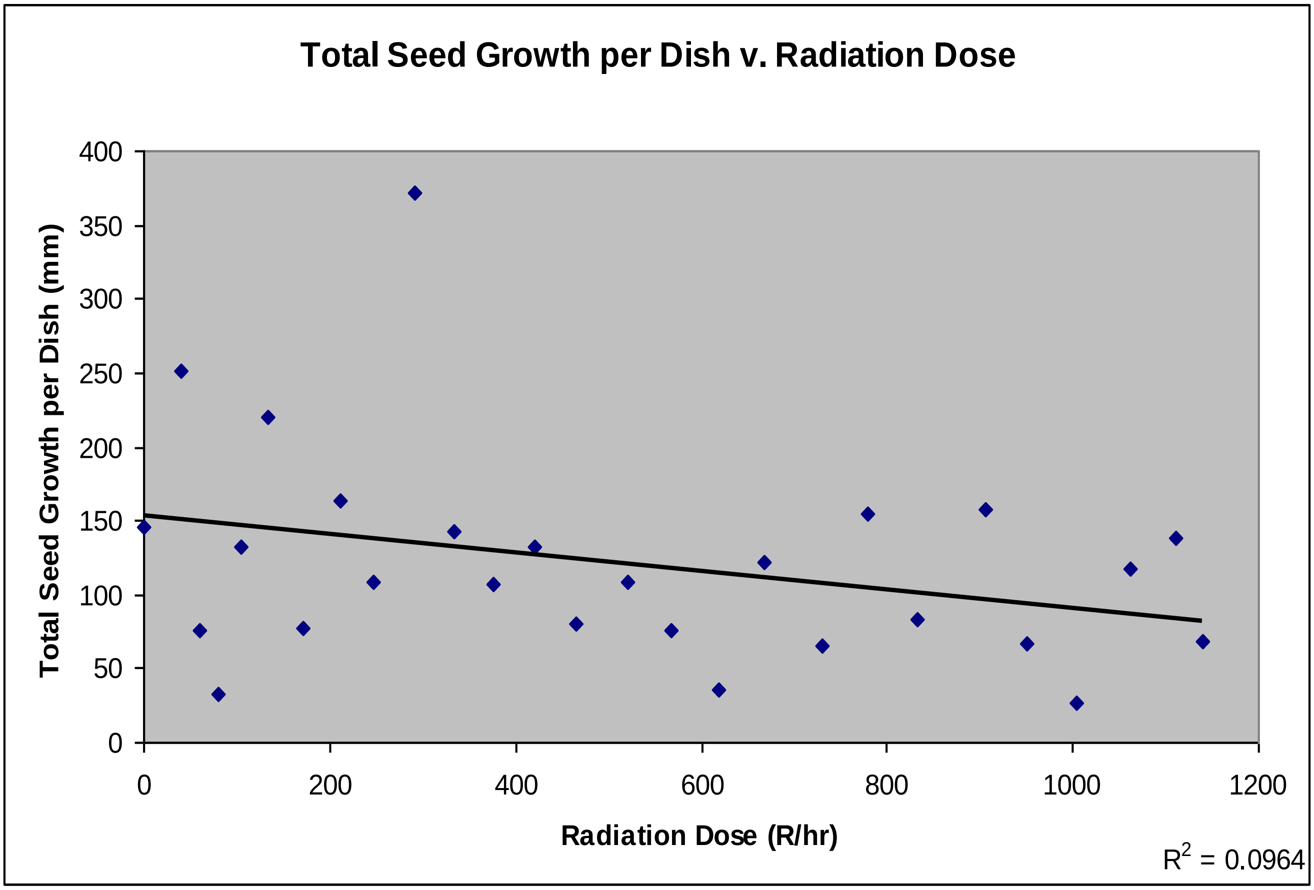


**Figure 3**

The average seedling growth per dish compared to the dose of radiation after 14 days of growth. There is a slightly negative slope (-0.003 avg growth in mm per (R/hr)), but it is not statistically significant according to a linear regression ($p = 0.116$, $N = 27$ dishes).

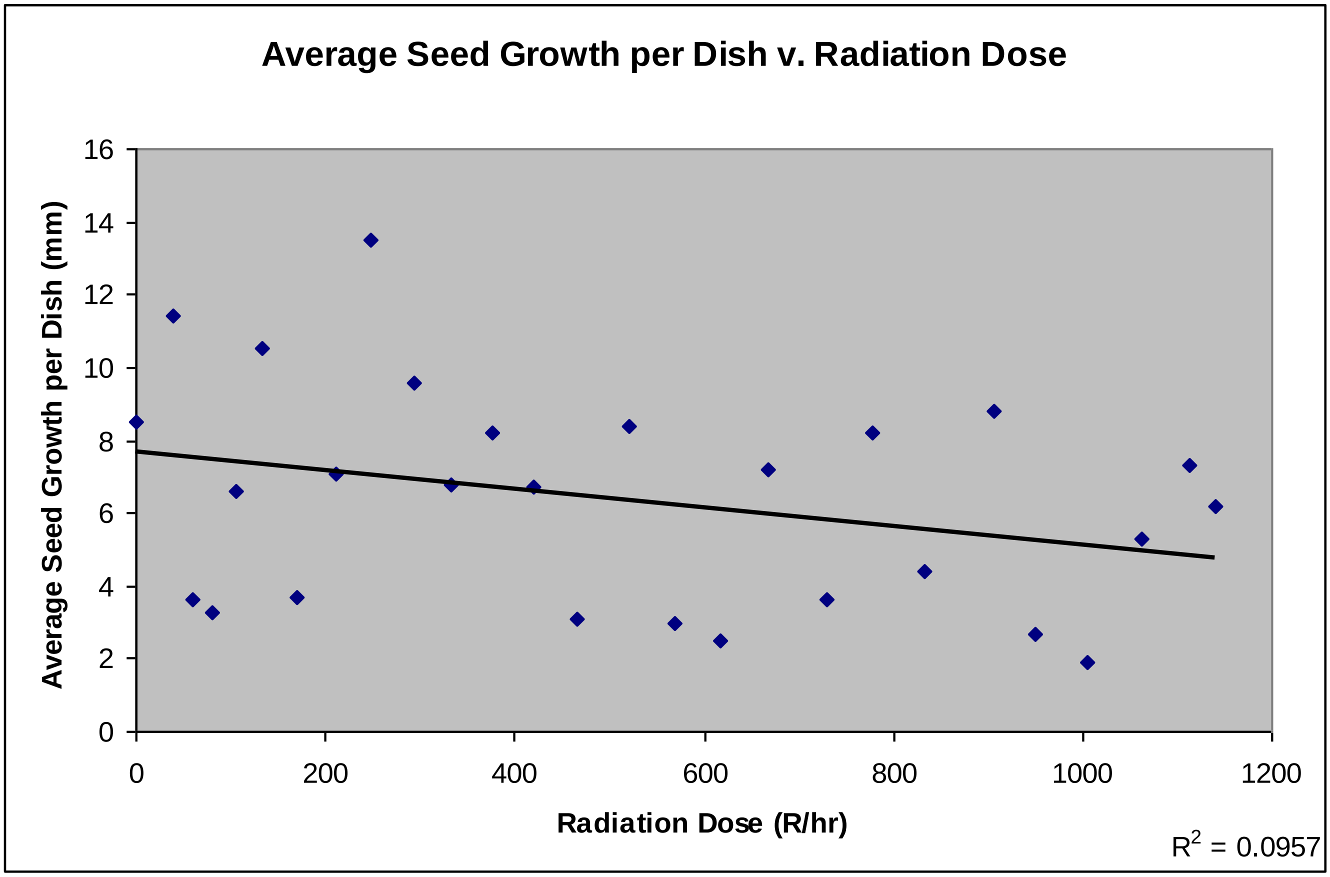


**Figure 4**

The total seedling growth per dish compared to the dose of radiation after 35 days of growth. There is a negative trend (-0.129 total growth in mm per (R/hr)), but it is not statistically significant according to a linear regression ($p = 0.042$, $N = 27$ dishes).

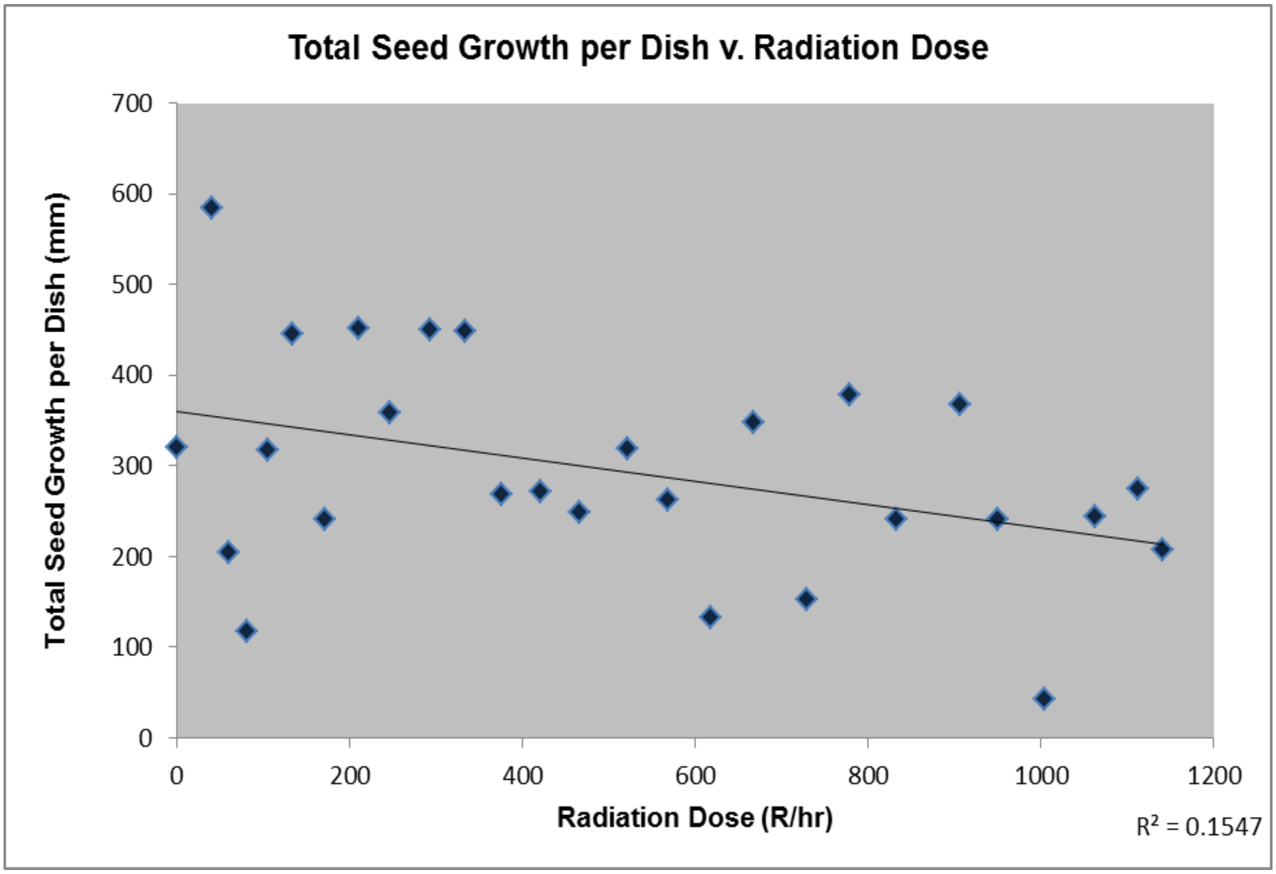
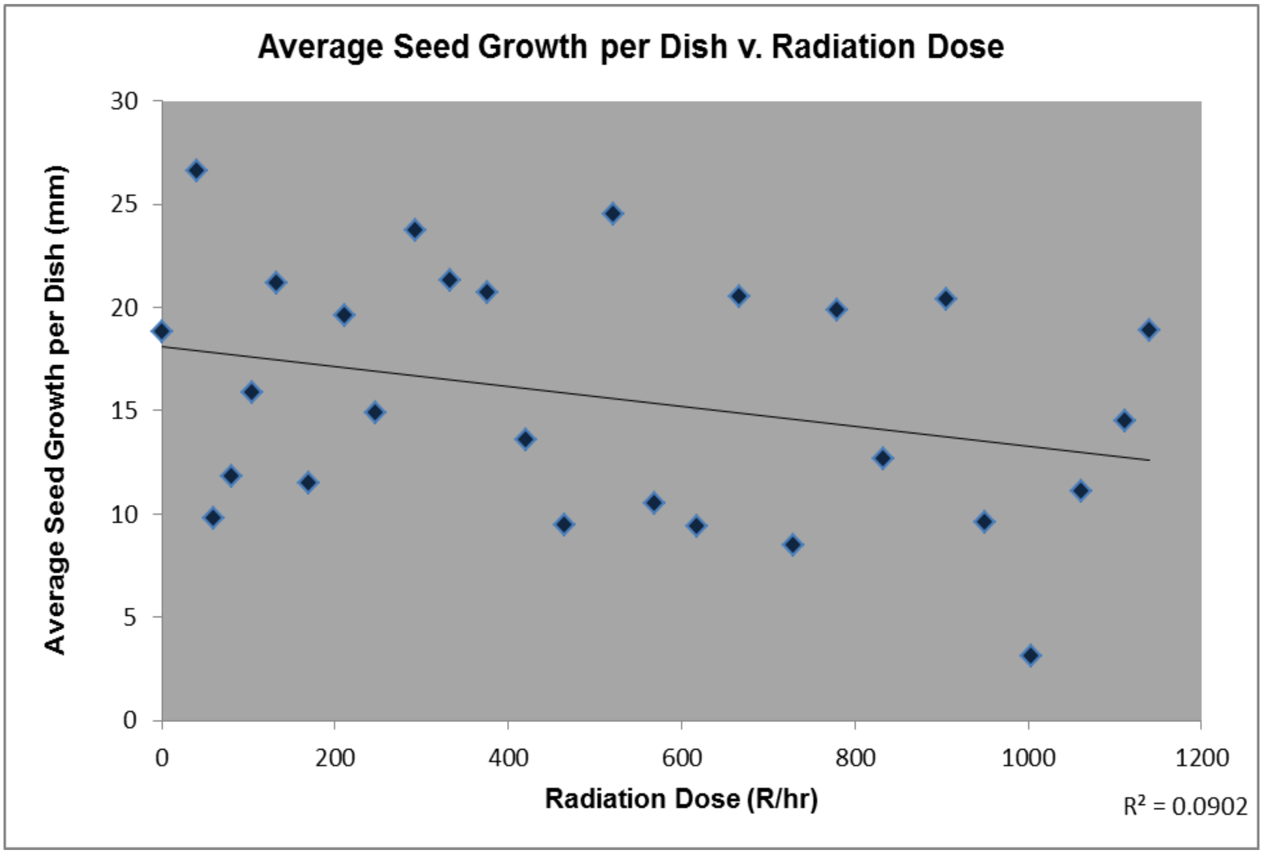


**Figure 5**

The average seedling growth per dish compared to the dose of radiation after 35 days of growth. There is a slightly negative slope (-0.005 avg growth in mm per (R/hr)), but it is not statistically significant according to a linear regression (p = 0.127, N = 27 dishes).



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# Endnotes

[1] Simak, Milan, and Ake Gustafsson. "X-ray photography and Sensitivity in Forest Tree Species." *Hereditas* 39, no. 3-4 (1953): 458-468. http://onlinelibrary.wiley.com/doi/10.1111/j.1601-5223.1953.tb03430.x/abstract (accessed March 19, 2012).

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[3] Op Cit.

[4] Wagenius, Stuart , and Stephanie Pimm Lyon. "Reproduction of Echinacea angustifolia in fragmented prairie is pollen-limited but not pollinator-limited." *Ecology* 91 (2010): 733-742. http://echinacea.umn.edu/pdf/wageniusAndLyon2010.pdf (accessed April 9, 2012).

[5] *Radiation Output Data MX-20*. Wheeling: Faxitron X-Ray Corporation, 2011.

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[8] "Calculate Your Radiation Dose | Radiation Protection | US EPA." *US Environmental Protection Agency*. N.p., n.d. Web. 8 Apr. 2012. <http://www.epa.gov/rpdweb00/understand/calculate.html>.

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